Proposal To:	California Department of Food and Agriculture Pest Exclusion Branch Nursery, Seed, and Cotton Program Attn: Katherine Filippini/Phuong Lao 1220 N Street Sacramento, CA 95814 (916) 654-0435 IAB@cdfa.ca.gov
Title of Proposed Research:	Development of a biomarker for walnut juvenility
Proposed Duration:	1 year
Starting Date:	July 1, 2022
Total Amount Requested:	$19,890
Submitting Organization:	The Regents of the University of California - Davis
Department:	Plant Sciences
Principal Investigator:	*Patrick J. Brown* *One Shields Ave. MS-2* *530-752-4288* *pjbrown@ucdavis.edu*
Cooperating Personnel:	*Aaron Leichty* *USDA ARS* *800 Buchanan Street, Albany, CA 94710* *aaron.leichty@usda.gov*
Send Award Notice to:	*Office of Research – Sponsored Programs* *Awards Team* *1850 Research Park Drive Suite 300* *Davis, CA 95618-6153* *530-754-7700* *awards@ucdavis.edu*

Project Title: Development of a biomarker for walnut juvenility

Project Summary/Abstract – Walnut scions originating from material micropropagated in tissue culture show reduced yield, delayed catkin production, and distinct pigmentation compared to genetically identical field-propagated scions. We hypothesize that tissue culture imparts a semi-stable epigenetic state characterized by persistent juvenility. Grafted orchards established using mature scion material originally derived from tissue culture still show reduced yield, suggesting that the epigenetic changes associated with persistent juvenility can persist for a decade or more. We propose to investigate this phenomenon at the molecular level in order to develop a biomarker for walnut juvenility, which would allow material to be screened for persistent juvenility before being used for propagation.

Project's Benefits to the Nursery Industry – Improvements in walnut tissue culture technique over the last 20 years have allowed the industry to rapidly adopt clonal rootstocks. Micropropagation also has advantages for scion propagation: material can be rapidly increased throughout the year under sterile conditions, and can easily be rooted for production of own-rooted English walnuts for areas where cherry leaf-roll virus (CLRV) is a problem. However, discussion at the 2017 Walnut Research Conference provided the first inkling that micropropagated walnut scions might show reduced yield compared to "ordinary", field-propagated scions. Over the last two years, we have received numerous reports of Chandler orchards showing delayed catkin production and reduced yield, and in each case the scion material appears to have originated from material that passed through tissue culture. Many of these orchards have since been removed, but new reports of afflicted orchards keep coming in. This is specifically a problem for the nursery industry, since delayed catkin production and reduced yield may be less apparent in orchards maintained for propagation than in production orchards. We propose to develop a biomarker for walnut juvenility that could be used to screen scion material intended for propagation. Ultimately this knowledge could potentially be used to develop treatments for orchards with persistent juvenility, and/or to develop tissue culture techniques that avoid the problem in the first place.

Objective – To identify a biomarker for walnut juvenility by comparing mRNA and miRNA expression between micro-propagated and field-propagated Chandler scions.

Workplans and Methods – We propose to select three sites for sampling. Each site will include micropropagated and field-propagated Chandler trees in close proximity, either from replants in an orchard that originate from a different source of scion material, or from (less ideally) a neighboring orchard of similarly-aged Chandler. We know of one site where alternating pairs of rows consist of micropropagated and field-propagated Chandler trees, grafted onto clonal rootstock, established at the same time and managed identically. This site will be prioritized for sampling. Additional sites may be sampled, and analyzed using other funding sources. At each site, three trees each of micropropagated and field-propagated Chandler will be sampled at two time points: once during the growing season and once during the dormant season, for a total of 12 samples per site or 36 samples total. Biomarkers detectable during the growing season and the dormant season would be practical for budwood and graftwood testing respectively, and we hope to identify a single biomarker that could be used throughout the year.

a. *Brief task objective(s): to identify a pattern of messenger RNA (mRNA) or microRNA (miRNA) expression that is perfectly correlated with walnut juvenility across all of our samples.*

b. *Activities and methods description:6 trees per site (3 each micropropagated and field-propagated) will be marked with paint. Axillary buds will be collected twice from each site, once in August 2022 and once in January 2023. Buds will be flash frozen in liquid N and transported back to the laboratory. mRNA and miRNA extraction, library preparation, and sequencing will be performed at the UC Davis Genome Center, and data analysis will be performed by the PI.*

c. *Task products and estimated completion dates: Sample collection will be completed by January 2023. Data analysis will be completed by June 2023.*

Project Management and Evaluation – Pat J. Brown will coordinate sample collection and analysis, assisted by undergraduate assistants supervised by permanent staff of the Walnut Improvement Program. Two key cooperators will provide intellectual input: Wes Hackett, who has studied juvenility for many decades in Hedera Helix (English Ivy) and other model plants during his long horticultural career; and Aaron Leichty, a new USDA employee focused on California tree crops who has expertise in molecular mechanisms of juvenility in plants. Success of this project will be assessed by the degree to which walnut juvenility across different sites, tissues, and seasons can be associated with common molecular signatures.

Literature Review – The progression from the juvenile to the adult phase in higher plants (including monocots and dicots, annual and woody species) is associated with changes in micro RNAs, which are small RNAs that do not encode proteins but rather exert their effects by regulating the expression of other genes (Wang et al., 2011). Specifically, the juvenile phase in plants is associated with high expression of miR156 and low expression of miR172, and the adult phase in plants is associated with the opposite pattern. Previous in silico analyses have identified putative miRNA transcripts in the Chandler genome, and several previous empirical studies have characterized miRNA expression in Chinese walnut cultivars. To our knowledge no empirical data have previously been collected on miRNA expression in Chandler or other California cultivars.

Wang, J.-W., Park, M.Y., Wang, L.-J., Koo, Y., Chen, X.-Y., Weigel, D., and Poethig, R.S. (2011). MiRNA Control of Vegetative Phase Change in Trees. PLOS Genet. *7*, e1002012. https://doi.org/10.1371/journal.pgen.1002012.

Current and Pending Support – *NA*

IAB – Budget Proposal Template

Project Title/Description:

Project Leader:

Proposed Fiscal Year:

A. PERSONNEL SERVICES:

Undergrad Student Asst.	@$15/hour x	280 total hours	$4,200
Staff Benefits	= 1.9%		$80
TOTAL PERSONNEL SERVICES:			$4,280

B. OPERATING EXPENSES:

Laboratory Supplies	$3,000
Travel (per diem)	$500
Postage	$
Other: (sequencing costs)	$11,682
TOTAL OPERATING EXPENSES:	$19,462

C. INDIRECT COST: $428

D. TOTAL BUDGET REQUESTED: $19,890

*Round dollar amount to the nearest dollar
*Type out acronym "FTE"
*Make sure % and dollar amount add up

Budget Justification

Key/Senior Personnel
Patrick J Brown, Principal Investigator: no funds requested

Other Personnel
Undergraduate Student Assistant: 280 hours at $15/hour to assist with sample collection and lab analysis

Fringe Benefits
The UC Davis composite benefits rate for undergraduate student titles is 1.9%. Total = $80

Travel
$500 is requested for travel between research plots and main campus.

Materials and Supplies
$3,000 is requested for lab and field supplies including tips, tubes for nucleic extraction, and molecular biology reagents.

Other Costs
Costs for tagseq sequencing is 36 samples x $104/sample = $3,744
Costs for miRNAseq sequesncing is 36 samples x $93/sample = $3,348 + library prep. $660 fixed cost + $1,965*2 fixed cost = $7,938
Total = $11,682

Indirect Costs
Indirect costs are 10% of personnel (salary + benefits) = $428